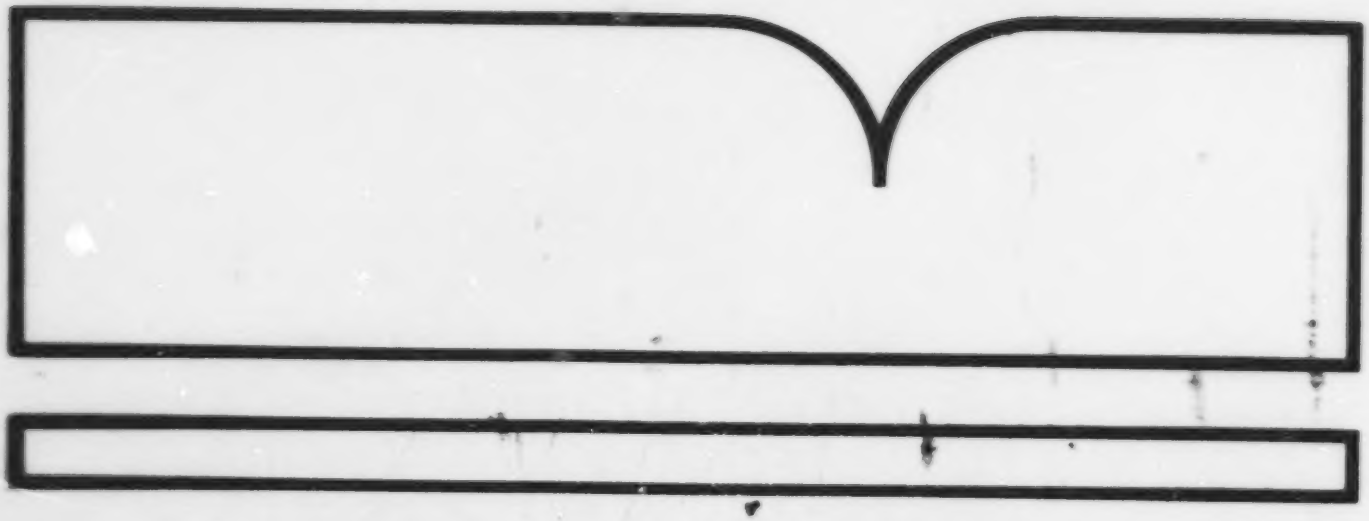
PB83-149310

Reduced Weight in Mice Offspring After In Utero Exposure to 2450-MHz (CW) Microwaves

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

30 Nov 81



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1. REPORT NO.	2.	3.
EPA-600/J-82-183	JOURNAL	PB83-149310

4. TITLE AND SUBTITLE	5. REPORT DATE
JOURNAL ARTICLE: Reduced Weight in Mice Offspring After In Utero Exposure to 2450-MHz (CW) Microwaves	1982
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) E. Berman, H.B. Carter, and Dennis House	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Experimental Biology Division and Data Management Office Health Effects Research Laboratory, US Environmental Protection Agency, Research Triangle Park, NC	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Office of Research and Development Health Effects Research Laboratory US Environmental Protection Agency Research Triangle Park, NC 27711	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

Published in: Bioelectromagnetics 3:285-291 (1982)

16. ABSTRACT

Time-bred CD-1 mice (100) were sham-irradiated or irradiated with 2450-MHz (CW) microwaves at 28 mW/cm^2 for 100 minutes daily from the 6th through 17th day of gestation. The offspring were examined either as fetuses after hysterotomy on the 18th day of gestation or as naturally born neonates on the 1st and 7th day of age. Fetuses of half of the dams were examined on the 18th day of gestation. The incidence of pregnancy and the numbers of live, dead, resorbed, and total fetuses were similar in both groups. The mean weight was significantly lower in live microwave-irradiated fetuses, and ossification of sternal centers was significantly delayed. In the offspring that were born naturally, the mean weight of microwave-irradiated 7-day-old suckling mice was significantly lower (10%) than that of the sham-irradiated group.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

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NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to Public	Unclassified	8
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

EPA-600/J-82-183
JOURNAL ARTICLE

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Reduced Weight in Mice Offspring After In Utero Exposure to 2450-MHz (CW) Microwaves

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Time-bred CD-1 mice (100) were sham-irradiated or irradiated with 2450-MHz (CW) microwaves at 28 mW/cm^2 for 100 minutes daily from the 6th through 17th day of gestation. The offspring were examined either as fetuses after hysterotomy on the 18th day of gestation or as naturally born neonates on the 1st and 7th day of age. Fetuses of half of the dams were examined on the 18th day of gestation. The incidence of pregnancy and the numbers of live, dead, resorbed, and total fetuses were similar in both groups. The mean weight was significantly lower (10%) in live microwave-irradiated fetuses, and ossification of sternal centers was significantly delayed. In the offspring that were born naturally, the mean weight of microwave-irradiated 7-day-old suckling mice was significantly lower (10%) than that of the sham-irradiated group. Survival rates of neonates in these two groups were not different. These data demonstrate that the decreased fetal weight seen in microwave-irradiated mice is retained at least 7 days after birth. Evidence from other published studies is presented to show that the retarded growth is persistent and might be interpreted as permanent stunting.

Key words: mice, microwaves, body mass, development

INTRODUCTION

It has been stated that one of the most consistent effects seen in the rodent fetus as a result of exposure to microwave radiation is decreased weight [O'Connor, 1980]. On its own, a temporary decrease in fetal weight (ie, a delay in growth) may not cause much interest in microwaves as a teratogenic agent. But, a chronic low weight (ie, stunting) is considered an important index of neonatal condition [Hurley, 1977]. Therefore, resistent lowered weight after birth would represent a more severe demonstration of the teratogenic potential of microwaves than would only delayed growth in the fetus. To determine which of these two conditions results from microwave exposure, we chose

Received for review January 13, 1981; revisions received September 22, 1981 and November 30, 1981.

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0197-8462/82/0302-0285$02.50 1982 Alan R. Liss, Inc.

a regimen of treatment that we were reasonably sure would cause decreased fetal weight in mice [Berman et al, 1978], and we also examined the offspring's weight at 7 days of age.

MATERIALS AND METHODS

All animals used in this study were CD-1 mice (Charles River Laboratories, Wilmington, Mass). Time-bred, primipara female mice were received from the supplier and randomly assigned two per cage as they were removed from the shipping crate. Assignment to experimental groups was done by cage-pairs. Each pair was first assigned to one of 25 exposure-array positions for the duration of the daily exposures. Assignment to one of four groups was done on the basis of position in the exposure array, where the pair in Position 1 was either sham-irradiated or microwave-irradiated, and their fetuses examined near term. The pair in Position 2 were either sham- or microwave-irradiated, allowed to give birth, and their neonates examined. Assignments were made for each of the 25 exposure positions using this alternating basis.

In the first replicate, the 25th position was assigned to the group whose fetuses were examined; in the second replicate, the animals in the 25th position were included in the group allowed to give birth. Treatment consisted of microwave- or sham-irradiation for a period of 100 minutes daily from the sixth through the 17th day of gestation. Experimental treatments occurred between 1000 and 1500 h.

Experimental methods have been reported in detail [Berman et al, 1978]. Briefly summarized: 25 bred mice, in individual vented plastic cages, were put into a controlled-environment chamber constructed of foamed polystyrene. The chamber was situated in a microwave anechoic chamber and was irradiated by CW 2450-MHz energy at a power density of 28 mW/cm^2. The ambient temperature was 20.2 ± 0.5 °C, the relative humidity was 50 ± 5%, and the air flow, 0.1 m/s. The sham-irradiated animals were treated in a foamed-polystyrene environmental chamber adjacent to the anechoic chamber; the temperature-controlled air was circulated through both environment chambers so that the ambient air was common to both groups. The study was done as two replicates, each with 25 bred mice in each treatment group, which resulted in totals of 50 bred mice in the sham-irradiated group and 50 in the microwave-irradiated group. Each group of 25 mice was exposed in a 5 by 5 array of animals. In such an array, the specific absorption rate (SAR) as determined by twin-well calorimetry is 0.59 ± 0.16 ($\bar{X}$ ± SD) mW/g per 1 mW/cm^2 of incident radiation; at the incident power density used in this study (28 mW/cm^2), the mean SAR is 16.5 ± 4.5 mW/g.

On the 18th day of gestation, half of the sham- and microwave-irradiated females were killed. The uterus of each dam was examined for signs of pregnancy and counts made of live, dead, and resorbed fetuses. Individually, the live fetuses were blotted dry, weighed, and fixed in ethyl alcohol. Later, these fetuses were macerated and cleared with a 1% KOH solution and their skeletons stained with Alizarin red-S so that the sternal ossification centers could be seen.

The remaining half of the microwave- and sham-irradiated dams were allowed to deliver their young. Only data on litters that were born on the 20th day of gestation are included in the results. Live and dead neonates were counted the morning after birth. On the seventh day of age, live offspring were counted again and weighed as a litter. Culling to equal litter size was not done.

Since the litter was used as the experimental unit, the statistical analyses of weight and number of sternal ossification centers was done using the mean value per fetus of

TABLE 1. Incidence of Pregnancy and Fetal Data (Analyzed by Litter) for CD-1 Mice

	Exposure	
	Sham	Microwave[a]
Bred females	22	21
Pregnant	14	17
Live fetuses[c]	10.1 ±2.4	11.1 ±2.0
Dead or resorbed fetuses[c]	0.79 ±0.89	0.41 ±0.62
Sternal ossification centers[c]	5.91 ±0.28	5.69[b] ±0.31

[a]Exposed to 2,450-MHz microwaves at 28 mw/cm^2 100 minutes daily from 6th through 17th day of gestation.
[b]$F = 4.19$; $df = 1.29$; $P = .049$.
[c]Values are mean ± SD.

TABLE 2. Numbers of Living and Dead of Neonatal Offspring of CD-1 Mice*

	Exposure	
	Sham	Microwave[a]
Number of litters	12	10
On day 1:		
Born alive	10.0 ±1.7	10.8 ±1.1
Stillborn	0.42 ±0.79	0.09 ±0.30
On day 7:		
Alive	9.83 ±1.99	10.70 ±0.95
Died since birth	0.58 ±0.90	0.30 ±0.67

*Observations Made on day 1 and day 7 postpartum. Values are mean ± SD for litters.
[a]Exposed in utero to 2,450-MHz microwaves at 28 mW/cm^2 for 100 minutes per day on days 6–17 of gestation.

these variables calculated on each litter. Litter size, number live, and number dead were analyzed using the appropriate count for each litter as the data. Analyses were done using computer programs of the Statistical Analysis System [Helwig and Council, 1979].

RESULTS

Analysis of variance for a one-way model revealed no significant differences in litter sizes or in the numbers of alive or dead fetuses or pups between sham- and microwave-irradiated groups at 18 days of gestation, at birth, or at 7 days of age (Tables 1 and 2). The difference in the means of the average number of sternal ossification centers per fetus (Table 1) were barely significant when analyzed using a one-way ANOVA model ($P = .049$).

Because the variance of the mean weight of neonates at 7 days of age was unequal to (19 times larger than) the variance of the mean fetal weight at 18 days of gestation

TABLE 3. Litter Mean Weight (g) per Fetus and per Neonate (±SD) for Each Exposure and Age Group Combination

	Exposure Group (mW/cm^2)	
Age Group	0	28
18-day-old fetuses	1.10(±0.11)	0.99(±0.11)
	n=14	n=17
7-day-old neonates	4.32(±0.48)	3.89(±0.38)
	n=12	n=10

TABLE 4. Results of Analysis of Covariance for Transformed Litter Mean Weight(g) per Fetus or Neonate

Source of variation	DF	MS	F	P
Age	1	23.3609	2525	0.0001
Group	1	0.0963	10.4	0.0023
Age:group	1	0.0001	0.01	0.9234
Live	1	0.0807	8.73	0.0048
Error	48	0.0092		

(Table 3), a log transformation was done on the mean weight per neonate to help equalize variation. These transformed values were analyzed using an analysis of covariance for a two-way design. Group (sham- vs microwave-irradiated) and age (18-day-old fetuses vs 7-day-old neonates) were the two factors and live (the number alive in litters) was the covariate. The ANOVA results are in Table 4.

The regression coefficient for mean fetal weight by litter size of sham-irradiated litters is -0.029 ($P = .012$); for mean neonatal weight, the corresponding regression coefficient is -0.14 ($P = .036$). The analysis shows that the number alive is a significant source of variation, which has been removed in this analyses using covariance adjustment. The observed negative regression coefficient for number of live means that large litters have a small litter mean weight per fetus (and neonate) and vice versa. Age and group were both significant factors, while the interaction of the two factors was not significant. The lack of interaction means that age effects are consistent across groups or vice versa.

DISCUSSION

The potential that microwave radiation may have to cause teratologic effects has been frequently studied. Exposure durations have ranged from minutes on a single day of gestation to essentially the entire period of gestation. Variations of experimental design among these studies extend to observations of fetuses and neonates—even to maturity. Two consistent practices in these experiments are the measurement of weight and the almost exclusive reliance upon the rat and mouse as experimental subjects. Table 5 is a summary of the reports of weight measurements of mice irradiated by microwaves in utero. The table is arranged by the stage of development at which weight was measured and by carrier frequency of microwaves used in the exposure. Also included are the exposure duration(s) and day(s) of gestation in which the exposure(s) occurred and the trend of exposed subjects' weights in relation to those of sham-irradiated (or, if not reported, control) subjects'. The direction of this effect is a negative sign (−) for reduced

TABLE 5. Summary of Weight Data From Reported Experiments in Which In Utero Irradiation Occurred *

Frequency (MHz); and power density (mW/cm^2)	Exposure duration and timing	SAR[a] (mW/g)	Weighed on day of age and comments	Effect	S/NS	Reference
Fetuses						
2450; 3.4	100 min/d GD 1–17	2	near term	+	NS	Berman et al [1978]
3000; 10	2 h/d GD 1–15	8	near term	–		Bereznitskaya and Rysina [1973]
2450; 14	100 min/d GD 1–17	8	near term	–	NS	Berman et al [1978]
2450; 28	100 min/d GD 6–15	16.5	near term	–	S	This study
2450; 28	100 min/d GD 6–15	22	near term	–	S	Berman et al [1978]
2450;	2–5 min GD 8	3–8	near term; stunting	–		Rugh et al [1975]
Postpartum						
10 cm; 10	2 h/d GD 1–15	8	1 DA 3–21 DA	– –	S	Bereznitskaya and Rysina [1973]
10 cm; 10	2 h/d	8	exposed before and during gestation; 1–21 DA	–		Bereznitskaya [1968]
"SHF"; 50	10 min/d		exposed for 12 d of gestation; 1–21 DA	–		Pobzhitkov et al [1961]
2450; 28	100 min/d GD 6–15	16.5	7 DA	–	S	This study
2450;	4 min, GD 9, 12, or 16	22–27[b]	60 DA: all males : GD 9 or 12 females :GD 12 females	– – –	S NS S	Rugh [1976]

*Data are ordered by observations during or after gestation. Effect: + = irradiated subjects heavier than control subject, – = control subjects heavier than irradiated subjects, d = day(s), GD = day(s) of gestation. DA = postpartum day of age, S/NS: S = statistically significant difference, NS = not statistically significant.
[a]From publication or estimated from Durney et al [1978].
[b]Total energy absorbed is given in Joules.

weight of microwave-irradiated subjects or a plus sign (+) for increased weight. This symbol was assigned even if the authors reported only textual description of results, and even though statistical tests of the results may not have been described. Where description of statistical tests were described, notation of statistically significant (S) or not significant (NS) results are made in the table. The SARs in Table 5 are either from the cited reference or from estimates based on Durney et al [1978]. In the latter cases, the SAR (mW/g per mW/cm^2) was estimated as 0.8 at 2450 MHz from the data in Figure 38 (large mouse).

Of the reported experiments with mice irradiated with microwaves during gestation, most have resulted in a decrease of the offspring's weight. O'Connor [1980] has discussed the consistent trend of decreased fetal body weight after microwave irradiation but has left unanswered the question of postpartum persistence of deficits.

Berman et al [1978] observed a 10% decrease in fetal body mass of mice irradiated (2450 MHz, 28 mW/cm^2) for 100 minutes daily during most of gestation (Table 5). When

we repeated essentially the same experiment for this study, again a 10% decrease was observed (Table 3). All of the fetuses were apparently normal; no gross morphologic anomalies were seen. Some of the fetuses were visibly "small for age" (a term denoting a fetus obviously small for its supposed gestational age, but otherwise appearing normal). However, this condition when seen alone is not considered a valid example of frank terata.

The sternum is composed of a number of bones that arise initially from centers of ossification. The method that we used for maceration, clearing, and subsequent staining of calcified material in the fetal carcass allows for convenient visualization of the ossification centers of the sternum. Six sternal ossification centers are usually seen in the 18-day-old mouse fetus (Table 1). The maturity of the skeleton can be estimated from the time of appearance of the sternal ossification centers, where the appearance of the centers occurs caudally and within a period of a few days. The progression of appearance is rather rapid, and a difference in one or two centers may reflect a difference of approximately 1 day in skeletal maturity. In this study, the calcified sternal centers of the live fetuses were counted, and the litter means were determined. The sham-irradiated fetuses had a mean of 5.9 calcified centers, while the microwave-irradiated fetuses had 5.7 calcified sternal centers (Table 1). Statistical analysis of these values by ANOVA resulted in $P = .049$, a value just within the realm of conventional statistical significance, and is evidence of a shift to immaturity in the skeletons of the microwave-irradiated fetuses.

It is appropriate to consider statistical comparisons by ANOVA of litter mean weight per offspring between sham- and microwave-irradiated groups at the fetal age and at 7 days of age postpartum. However, the variances of body weight at these ages are unequal, one being 19 times larger than the other. The statistical analysis was done using log-transformed values of litter weight. Furthermore, since the litter mean weight per offspring (fetal or neonatal) is a function of litter size (Table 4), weight was adjusted for litter size in this analysis. The strong association between weight and age ($P = .0001$) is due to the gain made during growth to 7 days of age. The difference in groups (sham- vs microwave-irradiated) is seen both at 18 days of gestation and at 7 days of age ($P = .0023$). But, most importantly, the factor of growth has not erased the decreased weight seen in the microwave-irradiated litters ($P = .9234$). Therefore, the decrease in body weight is persistent, and stunting has occurred as a result of exposure to microwaves.

Table 5 contains evidence that strongly supports the view that the microwave-induced retardation of growth seen at 7 days of age in the mice of this experiment would have persisted even longer. All of the reported studies of mice that have been irradiated by microwaves in utero have also reported decreased body weight at weaning [Pobzhitkov et al, 1961; Bereznitskaya, 1968; Bereznitskaya and Rysina, 1973] and even to 60 days of age [Rugh, 1976]. We know of no reports of offspring of mice that had been microwave-irradiated in utero being heavier after birth than concurrent controls.

Studies in which offspring have been treated in utero and remain with their natural mothers after birth are not designed to separate a fetal or offspring effect from a maternal effect. Only studies in which treated and nontreated newborns are cross-fostered can a maternal influence be separated from a fetal effect. Cross-fostering was not done in this study. It is our suspicion, though, that the continued retardation of growth is inherent in the irradiated fetuses and not in the dams. The usual indices of a maternally caused effect are data on neonatal and maternal survival. In the results of this study, there was no

significant increase in neonatal mortality. Actually, fewer neonates died in the microwave-irradiated group than in the sham-irradiated group ($\bar{X}$ = 0.30 and 0.58, respectively) even though the microwave-irradiated group was the larger population (Table 2). No dams were killed by the microwave irradiation in this study or in similar study in which a higher mean SAR was used (22 mW/g) [Berman et al, 1978]. Rugh [1976] used experimental parameters that resulted in a significant incidence of deaths from overexposure to microwave radiation (approximately 25 J/g in 4 minutes); his data (Table 5) are from survivors of this severe exposure regimen. Therefore, only after severe overexposure to microwave radiation during gestation has an increased incidence of maternal or offspring mortality been associated with a continuing retardation of growth after birth.

Of the two questions, the persistence of this effect (decreased neonatal weight) is a more immediate question than is possible maternal causation. Experiments designed to explore small differences in weight of neonatal or adolescent (or older) mice often fail; variance of growth values tends to increase with age. Because of the increased variance, weight alone has not proved to be a good measure of growth. Further investigation of stunting associated with microwave exposure will require additional functional tests of maturation.

In conclusion, we have confirmed earlier results of decreased fetal weight of mice exposed repeatedly to microwave radiation during gestation. We have shown that the lowered fetal weight is associated with delayed skeletal maturation, and we have demonstrated that the retarded growth of pups persists, at least to the 7th day of age. We cite additional evidence from the literature of potential teratologic effects of microwaves and, in the light of same, believe that the retarded growth and stunting will persist to maturation.

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